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**United States Patent** [19]**Garner et al.**[11] **Patent Number:** **5,639,940**[45] **Date of Patent:** **Jun. 17, 1997**

- [54] **PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS**
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- [52] **U.S. Cl.** ..... 800/2; 435/69.1; 435/69.6; 435/69.7; 435/69.8; 435/71.1; 435/172.3; 514/44; 800/DIG. 1; 800/DIG. 4; 800/DIG. 6; 935/47; 935/48; 935/53; 935/55; 935/70
- [58] **Field of Search** ..... 800/2; 435/172.1; 435/172.3; 69.1, 69.6, 69.7, 69.8, 71.1; 536/23.1, 23.4, 23.5, 24.1

[56] **References Cited****U.S. PATENT DOCUMENTS**

4,873,316 10/1989 Meade ..... 530/412  
 5,304,489 4/1994 Rosen ..... 435/320.1

**FOREIGN PATENT DOCUMENTS**

88-00239 1/1988 WIPO .  
 90-05188 5/1990 WIPO .  
 91/08216 6/1991 WIPO .  
 92/11358 7/1992 WIPO .  
 92-11757 7/1992 WIPO .

**OTHER PUBLICATIONS**

Grosschedi et al., *Cell* 38: 647-658, 1984.  
 Hartwig et al., *J. Biol. Chem.* 266: 6578-6585, 1991.  
 Roy et al., *J. Biol. Chem.* 266: 4758-4763, 1991.

Lord et al., *Blood* 73: 166-171, 1989.  
 Bolyard et al., *Blood* 73: 1202-1206, 1989.  
 Danishefsky et al., *Biochim. Biophys. Acta* 1048: 202-208, 1990.  
 Bolyard et al., *Gene* 66: 183-192, 1988.  
 Rixon et al., *Biochemistry* 24: 2077-2086, 1985.  
 Farrell et al., *Biochemistry* 30: 9414-9420, 1991.  
 Lord, *DNA* 4: 33-38, 1985.  
 Whitelaw et al., *Biochem. J.* 286: 31-39, 1992.  
 Farrell et al. *J. Biol. Chem.* 269: 226-231, 1994.  
 Palmiter et al., *Cell* 41: 343-345, 1985.  
 Chung et al., *Adv. Exp. Med. Biol.* 281: 39-48, 1990.  
 Chung et al., *Biochemistry* 22:3244-3250, 1983.  
 Lee et al., *Journal of Controlled Release* 29: 213-221, 1994.  
*Chemical Abstracts* 115: Abstract No. 202768k: 526, 1991.  
 Wall, 1996, *Thermogenology* 45:57-68.  
 Houdebine, 1994, *Journal of Biotechnology* 34:269-287.  
 Genes, Lewin, ed., John Wiley and Sons, N.Y. pp. 87-96.  
 D.F. Hoshier, "Disorders of Blood Coagulation" in *Cecil Textbook of Medicine*, 18<sup>th</sup> Ed., Wyngaarden et al., eds., W.B. Saunders Co., Philadelphia, 1988. pp. 1060-1065.  
 Pursel et al., *Science* 244: 1281-1288.  
 Hennighausen, 1990. *Protein Expression and Purification* 1:3-8.

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Materials and methods for producing fibrinogen in transgenic non-human mammals are disclosed. DNA segments encoding A $\alpha$ , B $\beta$  and  $\gamma$  chains of fibrinogen are introduced into the germ line of a non-human mammal, and the mammal or its female progeny produces milk containing fibrinogen expressed from the introduced DNA segments. Non-human mammalian embryos and transgenic non-human mammals carrying DNA segments encoding heterologous fibrinogen polypeptide chains are also disclosed.

**33 Claims, 5 Drawing Sheets**

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